



Johnson, J. L. (2021). The predictive potential of circulating microRNA for future cardiovascular events. *Cardiovascular Research*, 117(1), 1-3. Article cvaa145. <https://doi.org/10.1093/cvr/cvaa145>

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The predictive potential of circulating microRNA for future cardiovascular events

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Non-coding RNAs have received considerable attention within the cardiovascular research field over the last decade, especially the short non-coding RNA class commonly termed as microRNA (miRNA, miR). MicroRNAs are approximately 18–22 nucleotides in length and predicted to post-transcriptionally control 60-90% of protein-coding genes through inhibiting translation or promoting degradation of target messenger (m)RNA^{1 2}. Importantly, a single microRNA can potentially target a large number of genes¹, explaining why there are far less microRNAs than target genes, and there is evidence that multiple targets exist within the same functional networks. As such, mature microRNA possess the potential to play a central role in regulating cellular function, while their spatial and temporal expression patterns provide clues to their function^{1 2}. Accordingly, microRNAs are considered fine-tuners of gene and protein expression profiles during the progression of atherosclerosis, a proposition supported by human pathological studies alongside findings from diseased animal models where select microRNA function or expression have been modulated³. Such approaches identified multiple microRNAs which may serve as therapeutic targets for the prevention of clinical events associated with atherosclerotic plaque destabilisation, such as myocardial infarction and stroke³.

However, a marked proportion of patients who suffer a major adverse cardiovascular event (MACE) will have been previously asymptomatic, and even patients with confirmed clinically-relevant atherosclerosis and receiving optimal medical therapy, will experience additional future clinical events⁴. There is consequently a pertinent need for biomarkers which can readily inform a patient's risk of experiencing atherosclerotic plaque rupture and a subsequent clinical event. Relatedly, stable microRNAs have been detected within circulating blood, either conjugated with lipoproteins, packaged within extracellular vesicles (such as microvesicles and exosomes), or as a result of cell leakage during cell injury or apoptosis⁵. Moreover, circulating microRNA levels are associated with cardiovascular disease risk factors including hyperlipidaemia, and there are examples of specific miRNAs correlating with atherosclerosis and myocardial infarction³.

In this issue of Cardiovascular Research, Escate and colleagues⁶ examine if microRNA can be deployed as predictors of atherosclerotic plaque progression and related clinical events in patients with familial hypercholesterolaemia (FH), although potentially applicable to all individuals with atherosclerosis. The authors profiled microRNAs within exosomes isolated from circulating blood of patients with FH which had suffered a major adverse cardiovascular event (MACE) during an 8 year follow-up, alongside relatives without genetic diagnosis of FH and MACE, all participants within the SAFEHEART cohort⁷. This discovery approach identified 42 differentially expressed microRNAs which were subsequently subjected to pathway analysis and revealed 17 exosome-isolated microRNAs which were related to bioinformatically-defined atherosclerosis processes, such as 'inflammatory response'. Further RT-PCR validation of the 17 identified microRNAs was undertaken within exosomes, microvesicles, and plasma from a larger cohort of patients. This validation stage elucidated 9 microRNAs which were consistently increased within the plasma and exosome fraction of FH patients which had experienced MACE during

an 8-year follow-up compared to non-FH individuals with no evidence of MACE. Due to the correlation between plasma microRNA concentrations and those detected within exosomes, the authors propose plasma microRNA levels can serve as a surrogate for exosome microRNA content.

Subsequently, the 9 identified microRNAs were retrospectively compared within the plasma of FH patients with MACE, without MACE, and non-FH/non-MACE individuals subjected to 8-year follow-up. While this refinement stage revealed elevated expression of all 9 microRNAs were discriminatory for FH patients, only significantly increased miR-133a plasma levels identified FH patients experiencing MACE. Further analysis supported the predictive power of miR-133a (alongside miR-200c and miR-339-3p) for incidence of future cardiovascular events, including sudden death, fatal and non-fatal myocardial infarction, unstable angina and cerebrovascular incidents, during 5, 8, and 10-year follow-up periods after entering the SAFEHEART cohort, and independent of other cardiovascular risk factors. The authors next deployed in silico analysis to identify 923 potential target genes of miR-133a, 42 of which were delineated in a biased fashion due to their association with cardiovascular disease related processes identified using bioinformatics software analysis. Through further in silico analysis, the transcription factors FOXL2 and DNAJB6 alongside the membrane receptor CD130 (a unit of the IL-6 receptor complex) were predicated, implying miR-133a regulates the Wnt/ β -catenin and IL-6 signalling pathways, respectively. Substantiating these propositions, the authors demonstrated increasing miR-133a exogenously in primary human macrophages or microvascular endothelial cells elevated mRNA expression of both CTNNB1 and IL6R.

The study by Escate and colleagues⁶ affords a further microRNA to add to the armamentarium of potential biomarkers for atherosclerosis progression and risk of an associated clinical event (as summarised within Figure and reviewed by³). Although the present study focussed on FH patients (the most common genetic disorder associated with premature atherosclerosis-related cardiovascular disease), the findings have likely broader utility to all individuals with atherosclerosis. Supporting evidence is provided by similar microRNA profiling clinical studies revealing that miR-133a plasma levels are increased in patients with angiographic-defined coronary artery disease (CAD) when compared to healthy subjects⁸ and those experiencing an acute myocardial infarction (AMI) relative to CAD patients without an AMI or healthy individuals⁹. In addition, intra-plaque concentrations of miR-133a are increased in carotid lesions deemed symptomatic relative to asymptomatic plaques¹⁰, indicating that elevated circulating levels of miR-133a are likely to derive from vulnerable and ruptured plaques.

However, miR-133a is considered a cardiac-specific microRNA primarily expressed within cardiomyocytes¹¹, and the elevated levels detected post myocardial infarction are speculated to originate from injured and dying cardiomyocytes^{9, 12, 13}. While in accordance with the study from Escate et al⁶, circulating levels of miR-133a display prognostic value for all-cause mortality in CAD patients which present with AMI, such predictive capacity correlated with high-sensitivity troponin T levels and therefore the extent of myocardial injury¹². Escate and colleagues do not clarify the cellular or tissue source of the elevated miR-133a detected within their study or define if the association between raised miR-133a levels and future MACE are causal. While they infer the increase is plaque-dependent, the above-mentioned studies argue otherwise and even suggest microRNAs released from the damaged myocardium after an AMI track to coronary plaques to exert subsequent biological effects¹³. An alternate hypothesis is that miR-133a is released from coronary plaques after rupture (not causal) and during their subsequent healing, a phenomenon known to be prevalent within culprit coronary atherosclerotic lesions¹⁴. This possibility would question the applicability of miR-133a as a therapeutic target, as retarding vascular smooth muscle cell growth and ensuing plaque healing, including the reformation of the protective fibrous cap, may accelerate another adverse clinical event. Indeed, 13% of individuals within the SAFEHEART cohort have evidence of previous clinical premature atherosclerosis⁷, while mouse studies have revealed a central role for miR-133a in vascular smooth muscle cell growth¹⁵.

The current study from Escate and colleagues alongside other research reveals miR-133a as a potential surrogate marker of previous plaque rupture and subsequent AMI, representing a vulnerable patient with a preponderance for future clinical events, particularly those with overt risk factors such as FH. Additional studies are warranted to identify the source of circulating miR-133a and its potential distal biological function, particularly if it is to be considered as a therapeutic target for cardiovascular disease prevention. Finally, larger profiling studies to substantiate miR-133a as a bona-fide biomarker for disease progression should be encouraged, alongside other circulating microRNAs proposed as prognostic biomarkers (see Figure).

ARTICLE INFORMATION

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Acknowledgments

None.

Sources of Funding

J.L. Johnson is supported by the British Heart Foundation (FS/18/1/33234).

Disclosures

None.

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FIGURE LEGEND

Figure title: MicroRNA expression within circulating blood of patients with atherosclerosis.

This diagram illustrates the elevated microRNAs identified through profiling approaches within circulating plasma samples (including exosomes) and peripheral blood mononuclear cells (PBMCs) of patients with clinical atherosclerosis. Blue-filled box indicates intra-plaque cellular sources of circulating microRNA. Green-filled box signifies the forms in which circulating microRNA are packaged and potential other tissue sources. Line-coloured boxes indicate the patient cohorts from within which the associated upregulated microRNAs were identified. Yellow highlighting shows elevated miR-133a has been verified in an independent study, while microRNA with grey highlighting have been independently reported to be up- and down-regulated.

